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A Viral Disease of the Ivory Barnacle, *Balanus eburneus*, Gould (Crustacea, Cirripedia)

LOUIS LEIBOVITZ[1] AND SASHA KOULISH[2]

[1]*Laboratory for Marine Animal Health, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 and Professor Emeritus, Department of Avian and Aquatic Animal Medicine, New York State College of Veterinary Medicine, Cornell University, and* [2]*Biology Department, College of Staten Island, City University of New York, 130 Stuyvesant Place, Staten Island, New York 10301*

Abstract. The known diseases of the Class Cirripedia are reviewed. A previously unreported viral disease of the ivory barnacle, *Balanus eburneus* is described. The results of light and electron microscopic examinations of viral-infected tissues of an ivory barnacle are reported. The pathologic alterations of the parenchymal tissues and the cellular lesions produced by the invasion and replication of the virus in parenchymal cells are described. The large mature enveloped icosahedral virion (mean length 222 nm and width 175 nm) conforms to the larger viruses of the *Iridovirus* group of the family Iridoviridae.

Introduction

The class Cirripedia, the only sessile group of crustaceans, includes many diverse marine animals. The most familiar Cirripedes are the barnacles, approximately two-thirds of which are free-living and economically important as fouling agents on ships, piers, and other submerged marine structures. Barnacles are also commensals on external surfaces of whales, turtles, fish, and other crustacea and marine animals. As foreign bodies on the surfaces of these animals, barnacles may interfere with normal physiological functions and produce traumatic injuries.

Several groups of barnacles are important parasites of marine animals, such as the Rhizocephala parasitizing decapod crustaceans. Some larger species are used for human food. In spite of their importance and the need for biological control of their adverse effects, little is known about diseases that affect the Cirripedes.

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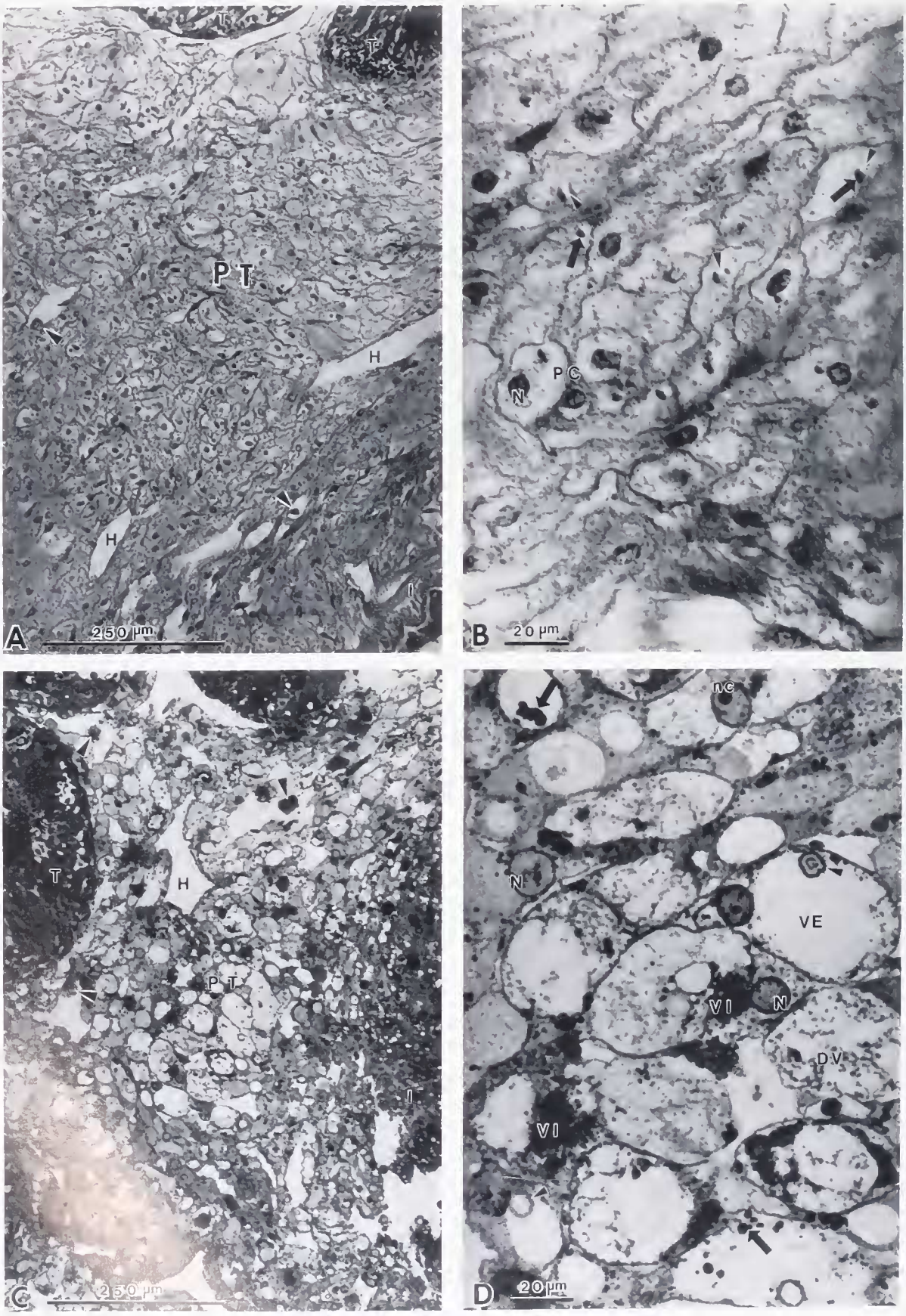
Naturally occurring diseases of Cirripedes are likely often overlooked due to the difficulties of observing signs of disease in a sessile animal covered by thick calcareous plates or existing in parasitic forms hidden within a host. Accordingly, descriptions of epizootic diseases or mass mortalities of Cirripedes have been rarely reported.

No bacterial or viral diseases of Cirripedes have been previously reported. Predators, commensals, and some parasites of Cirripedes have been described (Arvy and Nigrelli, 1969; Ching, 1978; Irwin and Irwin, 1980; Williams *et al.*, 1981). Epizootics, resulting in mass mortalities, have been observed periodically in marine laboratory barnacle populations maintained for biomedical research during a eight-year period (1981–1988) in Woods Hole, Massachusetts.

Previous work by Koulish, who conducted ultrastructural studies of the ivory barnacle, *Balanus eburneus* (Koulish, 1976; Koulish and Klepal, 1981; Barden and Koulish, 1983; Koulish and Gould, 1983; Koulish and Kramer, 1986), led to the research reported here, the description of the first known viral disease of the Cirripedia.

Materials and Methods

Ivory barnacles, measuring from 1 to 2 cm at their base, were obtained from the Marine Resources Center of the Marine Biological Laboratory in Woods Hole, Massachusetts, during May, 1986. No significant mortalities were noted in the laboratory's barnacle colonies at the time the barnacles were procured, and the animals appeared grossly normal. The barnacles were maintained in the laboratory in aerated artificial seawater at 20°C and fed periodically with invertebrate marine food (principally *Artemia nauplii*).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Barnacles selected for study were rapidly removed from their shells, cut into pieces (1–2 mm in the longest axis) on a cold plate and fixed for 3 h at 4°C in a 2.5% glutaraldehyde solution buffered to pH 7.2–7.4 with 0.1 *M* cacodylate containing 3% sucrose (Koulish and Kramer, 1986). Tissues were washed briefly in 0.2 *M* cacodylate buffer then post-fixed with 1% OsO_4 in the 0.1 *M* cacodylate buffer for 1 h, stained "en bloc" with 0.5% uranyl acetate (pH 3.9) for 30 min (Terzakis, 1968), washed in several changes of 0.2 *M* buffer then dehydrated in a graded series of alcohols, and embedded in Epon 812. Thick sections (1–1 μm) were stained with toluidine blue and examined by light microscopy.

Thin sections (80–100 nm) were collected on uncoated grids and stained with saturated aqueous uranyl acetate followed by lead citrate (Koulish and Gould, 1983). Observations were made at 75 kV with a Hitachi HU 11E electron microscope.

Among the specimens of *B. eburneus* examined, the parenchyma of one barnacle was found to be heavily infected with virus-like particles. The pathological alterations of the viral-infected parenchymatous tissues and cells and the details of viral replication were examined by light and transmission electron microscopy, photographed, recorded, and described.

Electron micrograph magnifications and measurements of viral particles were calibrated at three different magnifications by means of germanium-shadowed carbon replicas of a ruled diffraction grating, with a distance of 882 nm between the lines (Agar, 1967).

Results

Light microscopic examination

The "parenchymatous tissues" described by Tornava (1948) and Koulish (1976), surrounded and supported the visceral organs such as the gonads, ducts, and intestines in the mantle cavity. The hemolymph tissue spaces and vessels, which frequently contained hemocytes, traversed the parenchymal tissues (Fig. 1A). The normal parenchymal cells were pleomorphic interlocking cells that contained a variety of electron-dense and lucent cytoplasmic granules (Fig. 1B). In spite of their variable shapes, normal parenchymal cells were relatively uniform in size, except in close proximity to outer walls of visceral organs, where they formed a more dense adventitial coat of parallel compact cells. The latter cells were smaller, more elongated, and more darkly stained (Fig. 1A).

A "special" morphologically differentiated type of parenchymal cell containing many highly refractile zinc phosphate granules underlies the midgut region of barnacles and has been described by Koulish (1976).

In the virus-infected barnacles, histopathological lesions were limited to the parenchymal tissues. Cytopathic alterations of parenchymal cells were characterized by progressive disruption of cell structures (Fig. 1C). The nuclei showed margination and lysis of chromatin and hypertrophy of the nucleoli. The plasma membranes became distorted and poorly defined. Within the cytoplasm, dense granular collections and inclusion bodies were surrounded by lysed areas. The cytoplasmic granules, found normally in parenchymal cells, were frequently distorted, enlarged and enclosed in large vacuoles. Cellular edema and hypertrophy were pronounced. Inclusion bodies appeared to fragment and release their contents into the cleared cytoplasmic spaces and vacuoles. The nuclei of some infected cells were difficult to observe in the greatly swollen degenerated cells (Fig. 1D). Similar destructive changes were observed in individual hemocytes contained in hemolymph vessels and spaces within the parenchymatous tissues.

The architecture of the parenchymatous tissue became altered due to swelling, dissociation, displacement, and rupture of individual cells. Abnormal tissue spaces and irregularly shaped cords of cells formed (Fig. 1C, D). The adventitial parenchymatous tissues surrounding the vis-

Figure 1. Photomicrographs of parenchymal tissues and cells of uninfected and virus-infected ivory barnacles:

(A) Note the relatively uniform interlocking appearance of the uninfected parenchymal tissue (PT), the more elongated and compressed adventitial coat of parenchymal cells and testis (T), and the hemolymph spaces (H) containing hemocytes (arrows).

(B) Note the relatively uniform uninfected interlocking parenchymal cells (PC) and their nuclei (N), and the dark (large arrows) and light (small arrows) cytoplasmic granules.

(C) Note the disrupted architecture of the virus-infected parenchymatous tissue (PT), the irregularly shaped swollen vacuolated parenchymal cells, distended hemolymph spaces (H), containing swollen hemocytes (arrows), and the dark-staining adventitial cells containing many small refractile granules surrounding the intestine (I).

(D) Note the greatly enlarged virus-infected parenchymal cells with enlarged nuclei (N) and the nucleoli (nc), irregularly shaped cytoplasmic viral inclusion bodies (VI) and dispersed virions (DV), swollen light-staining granules (small arrows) and dark granules (large arrows).

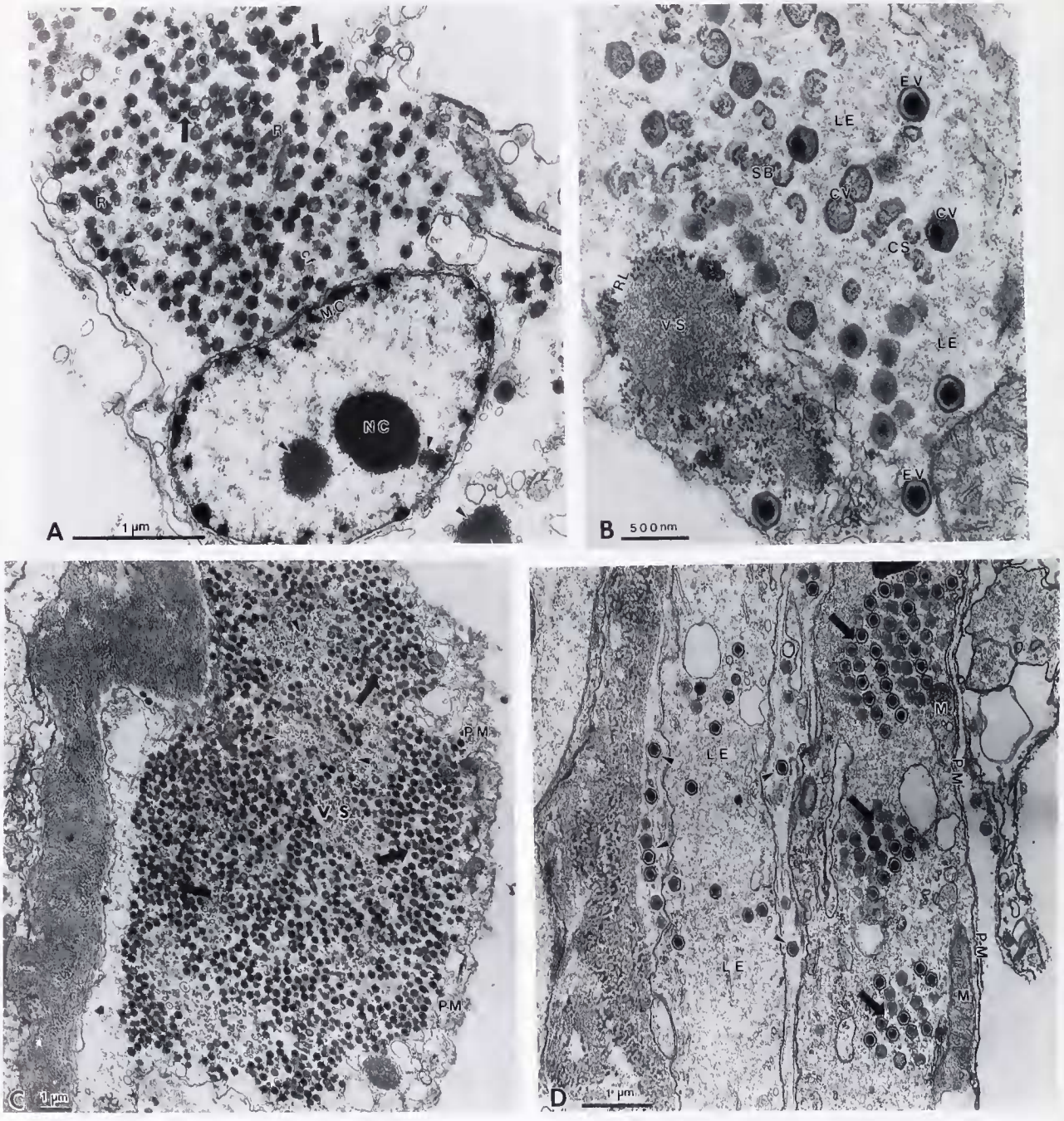

Figure 2. Electron micrographs of virus-infected parenchymal cells:

(A) Note the marginated chromatic (MC), the enlarged nucleolus (NC) with finely granulated loosely attached fragments within nucleus and cytoplasm (small arrows). Note the viral assembly in the cytoplasm, including rod-like forms (R), condensing-forms (cf), and non-enveloped virions (large arrows).

(B) Note the virogenic stroma (VS), lysed endoplasmic reticulum (LE), ribosomal-like particles (RL) assembled at the periphery of the virogenic stroma, crescent-shaped strands (CS), spiral-shaped brands (SB), condensing immature non-enveloped virions (CV), mature enveloped virions (EV), and mitochondrium (lower right).

ceral organs were less altered in shape but contained numerous highly refractile small colorless granules.

Transmission electron microscopic examination

In addition to the histopathologic changes observed by light microscopy, ultrastructural details of pathologic alterations of virus-infected cells and viral morphogenesis were obtained by transmission electron microscopy.

The light microscopic observations of nuclear hypertrophy, chromatin margination and lysis, and nucleolar hypertrophy were confirmed. In addition, nucleolar-like structures appeared to break up into finely granulated loosely aggregated fragments that were released within the nuclear and cytoplasmic compartments (Fig. 2A).

Viral replication and morphogenesis occurred at the periphery of the irregularly shaped granular electron-dense cytoplasmic inclusion bodies (virogenic stroma) in the cytoplasm of the parenchymal cells. Ribosome-like particles associated with the partially lysed endoplasmic reticulum were observed at the surface of the virogenic stroma (Fig. 2B). Virionic components appeared to assemble along the periphery of the stroma.

Based upon the apparent stages of development, viral morphogenesis began at the periphery of the viroplasm with the formation of fine filaments and the assembly of small crescent-shaped bilaminated strands within the disrupted endoplasmic reticulum (Fig. 2B).

The strands appeared to fuse and increase in length, forming long spirals that coalesced into less electron-dense oval or hexagonal outlined bodies, ranging approximately from 115 to 192 nm in length, and 92 to 154 nm in width. As the assembly process continued, the rounded bodies could be recognized as incomplete viral particles undergoing further assembly (Fig. 2B).

Occasionally, membrane-bound stout rod-like structures were observed in assembly areas (Fig. 2A).

The virionic assembly appeared to continue as the core or nucleus of the particle became more electron-dense and surrounded by a capsid. The viral assembly process extended throughout the mass of viroplasm, where all stages of assembly could be observed simultaneously. However, the greatest number of mature enveloped virions were found at the periphery of the vironic mass (Fig. 2C). With the exception of the adventitial parenchymal cells, virions were more widely dispersed within the cytoplasm of parenchymal cells evidencing marked degeneration and necrosis.

Upon completion of the viral assembly process in adventitial parenchymal cells, the mature virions were arranged in small arrays within the cytoplasm of the more elongated and compressed cells and could also be found in the intercellular spaces (Fig. 2D).

The distribution of these small viral arrays corresponded to the deeply staining granules seen in the adventitial parenchymal cells at the light microscope level.

The nucleocapsid ranged from approximately 134 to 154 nm in length, and 106 to 110 μm in width. The mature enveloped virions had a calculated length ranging from 184 to 253 nm, with a mean of 222 nm; and a width ranging from 147 to 216 nm, with a mean of 175 nm. The viral envelope, consisting of an inner matrix, a middle lipid bilayer, and an outer layer of peplomers could be seen surrounding the mature virions (Fig. 3).

Discussion

The disease is the first reported viral infection of barnacles or of any other member of the class Cirripedia. The viral agent had a predilection for parenchymatous cells of the barnacle, invading and replicating within these cells. Hypertrophy, degeneration, and necrosis of the parenchymatous cells and tissue resulted from the infection.

Although these cells have been designated as parenchymal cells, their exact function remains unknown. The term "parenchymal cells" has been reserved for the distinguishing or specific tissue cells of a gland or organ, as opposed to cells which form the supporting structure or stroma (Taylor, 1946).

While the parenchymal cells fill the spaces between the organs in the mantle cavity of barnacles, their function appears to be more than those of connective tissue. The relationship of parenchymal to hematopoietic tissues in barnacles has been a subject of speculation (Walley, 1969). The relationship of hemocytes to parenchymal cells is further suggested by the detection of viral-infected hemocytes in this study. Although some of the hemocytes were infected, the importance of hemolymph infection during the course of the disease was not established. The specialized (morphologically differentiated) types of parenchymal cells, indicated in this and other studies,

(C) Virogenic stroma (inclusion body) (VS) in cytoplasm of an adventitial parenchymal cell. Note the concentration of mature virions at the periphery of the stroma (large arrows) and immature virions in the process of assembly within, and the degenerating plasma membrane (PM).

(D) Note the small viral arrays (large arrows) within the cytoplasm of adventitial parenchymal cells, virions dispersed in the lysed endoplasmic reticulum (LE) and intercellular spaces (small arrows), the degenerating plasma membrane and adjacent mitochondria.

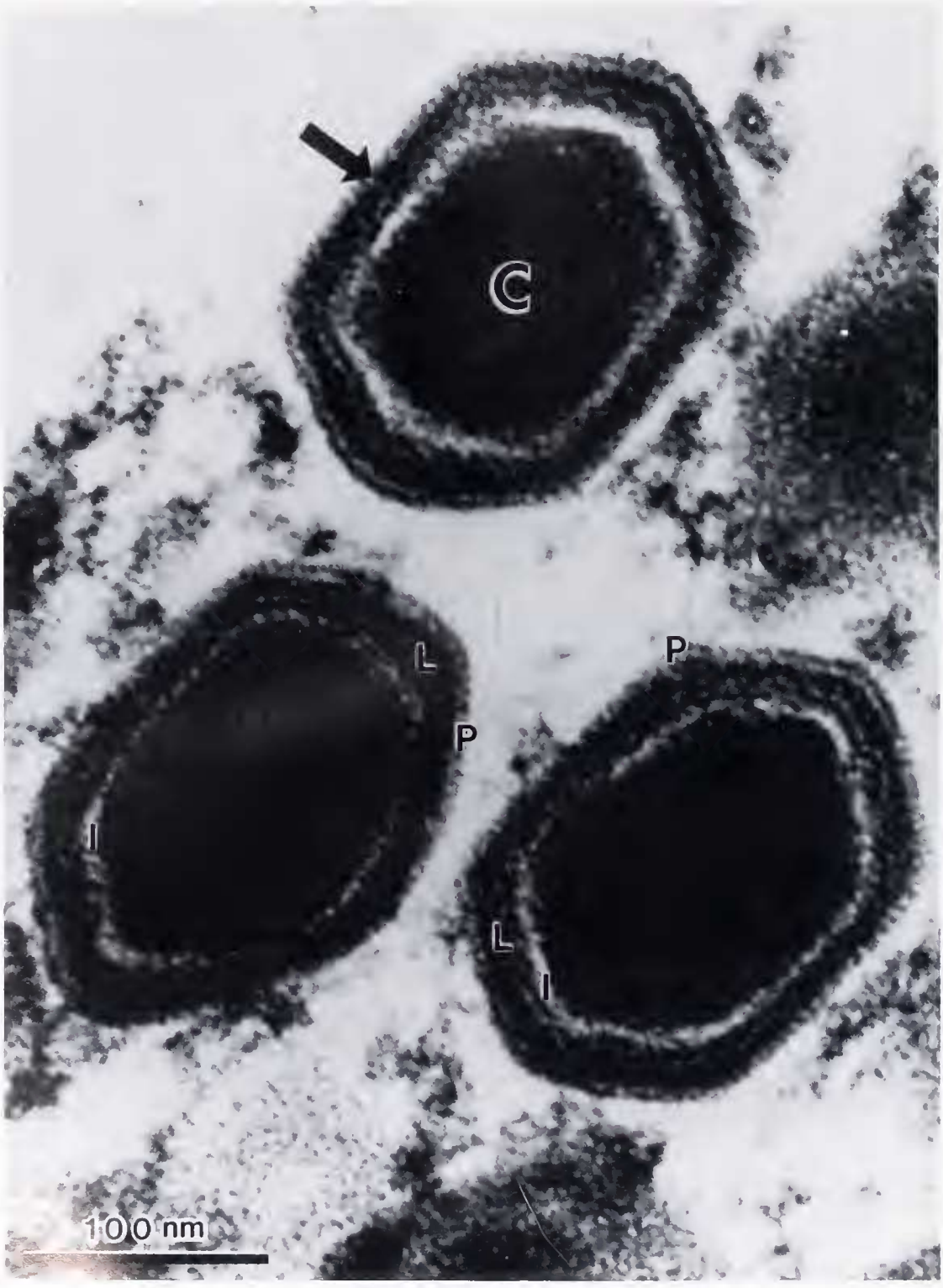

Figure 3. Mature hexagonal (icosahedral) enveloped virions (arrow) within the cytoplasmic space of [illegible]cted parenchymal cell. Note the nucleocapsid core (C) and its capsomeres, the well defined envelope, consisting of an outer layer of peplomers (P), a middle lipid bilayer (L), and an inner matrix layer (I).

suggest a variety of functions for these cells, which require further studies.

The morphological characteristics of the virion, their dimensions, and the method of viral replication and assembly, in close association with the cell's plasma membrane and mitochondria, conform to those reported for the family Iridoviridae, the largest of the cytoplasmic icosahedral DNA viruses (Hess, 1981; Fraenkel-Conrat and Kimball, 1982; Willis, 1985; and Fenner *et al.*, 1987).

Of the known iridovirus infections of arthropods, infections have been frequently reported in insects (Kelly and Robertson, 1973; Carey *et al.*, 1978); and in crustaceans, including isopods (Federici, 1980), and branchiopods (Frederici and Hazard, 1975).

Iridovirus infections have also been reported in mammals (Fenner *et al.*, 1987).

More studies are needed to determine the epizootiology, incidence, pathogenesis, comparative pathology, and the relative importance of this viral disease of barnacles and other members of the class Cirripedia.

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